Meeting report

The many layers of immunity

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A report on the Cologne Spring Meeting 'Immunity', Cologne, Germany, 13-15 March 2002.

The Cologne Spring Meeting organized by the Institute for Genetics in Cologne covers a different topic each year. This year's meeting on the theme 'Immunity' consisted of 22 talks over two and a half days. The meeting dealt with all aspects of immunity, ranging from cell-autonomous immunity, which functions in all nucleated cells, to the adaptive immune system that depends on B and T cells. It is becoming increasingly clear that the immune system has many layers, all of which are essential and highly interconnected. Here, I summarize some representative talks on different aspects of immunity.

Memory

Antonio Lanzavecchia (Institute of Biomedicine, Bellinzona, Switzerland) addressed the question of how memory is maintained in the adaptive immune system. In particular, he focused on the role of cytokines derived from dendritic cells (DCs) - tumor necrosis factor (TNF) α, interleukin (IL)-6, IL-10 and IL-12 - in promoting the proliferation of $CD4^+$ T cells in response to γc cytokines, which bind to receptors that share the γ common (γc) signaling chain, namely IL-2, IL-4, IL-7 and IL-15. Lanzavecchia reported that sensitivity to cytokines, and expression of cytokine receptors, vary with the differentiation stage of T cells. Naïve human $CD4^+$ T cells progressively acquire responsiveness to IL-7 and IL-15 and upregulate the IL-2/IL-15 receptor β chain while differentiating to central memory T (T_{CM}) cells or effector memory T (T_{EM}) cells. In addition, IL-7 and IL-15 act synergistically on all T-cell sub-populations but only T_{EM} can directly proliferate in response to these cytokines. In contrast, naïve and T_{CM} cells also need DCs or DC-derived cytokines in order to upregulate their relevant receptors. Lanzavecchia emphasized the striking difference in the response of naïve T cells and T_{CM} to cytokine stimulation. Cytokine-expanded naïve T cells maintain a lymph-node homing phenotype ($CD45RA^+$, $CCR7^+$) and undergo only limited differentiation. In contrast, T_{CM} cells acquire a phenotype resembling that of T_{EM} cells by downregulating CCR7 and upregulating CCR5 cytokine receptors. Like T_{EM} cells, these cells produced high levels of interferon (IFN) γ and IL-4, indicating that T_{CM} populations can generate cells resembling T_{EM} cells in an antigen-independent manner.

Lanzavecchia proposed a model according to which T_{CM} cells not only self-renew but also generate short-lived T_{EM} cells. These T_{EM} cells leave the secondary lymphoid organs and reside in peripheral tissue, where they can immediately respond to antigen. This would give the T_{CM} population a stem-cell-like character, constantly replenishing the pool of short-lived peripheral T_{EM} cells, and would explain how a polyclonal repertoire of memory T cells is maintained. Lanzavecchia also briefly presented a model and supporting evidence for long-term antibody memory based on chronic nonspecific stimulation of memory B cells by bacterial products such as lipopolysaccharide (LPS). This model puts the principles of T- and B-cell memory maintenance on the same basis.

Autoimmunity

Diane Mathis (Harvard Medical School, Boston, USA) presented fascinating work on a mouse model of rheumatoid arthritis. Characteristic of this autoimmune disease is the specific destruction of the synovial joints caused by local inflammation and recruitment of neutrophils and macrophages. B and T cells are essential for development of the disease, although their exact role remains controversial. Furthermore, what causes the recruitment of inflammatory cells in this apparently joint-specific manner is unknown. Working with K/BxN mice, which are the product of mice transgenic for a T-cell receptor (TCR) known as KRN crossed to the spontaneous autoimmune diabetic mouse, Mathis and colleagues observed development of severe, spontaneous rheumatoid arthritis. It was subsequently

shown that the KRN TCR recognizes a peptide derived from the ubiquitously expressed glycolytic enzyme glucose-6-phosphate isomerase (GPI) when it is presented by major histocompatibility complex (MHC) class II molecules. T and B cells autoreactive against GPI are essential for manifestation of the autoimmune disorder. Astonishingly, however, transfer of either serum from arthritic K/BxN mice or anti-GPI monoclonal antibodies into healthy animals provokes arthritis within days, even when the recipients are devoid of lymphocytes. Using serum transfer into various knockout mice, Mathis and colleagues investigated the cause of the joint-specific inflammation by a ubiquitously expressed enzyme and which factors are involved in the attraction of inflammatory cells. They found that mice lacking the FcγRI, FcγRIII and FcεRIII immunoglobulin (Ig) receptors were completely resistant to induction of rheumatoid arthritis by injection of serum from arthritic K/BxN mice. She also showed that the complement system is an essential player in the progression to rheumatoid arthritis in these mice. Surprisingly, the 'alternative' pathway of complement activation, which is normally activated by bacterial surfaces, seems to be the critical initiating route, resulting in the formation of C5a, the proinflammatory product of cleaving complement protein C5, and in turn recruitment of leukocytes and in particular neutrophils to the joints.

Having established the important roles of FcRγ and the complement system in the development of rheumatoid arthritis, Mathis asked why an autoimmune reaction to GPI should result in specific inflammation of the articular surface of the joints. No joint-specific form of GPI was found, so the regional specificity of the disease must result from something else. Mathis found that GPI is somehow localized on joint surfaces and that it co-localizes there with IgG and also - in a conjunction unique to this site - with the complement component C3. This may mean that GPI-IgG complexes also form in extra-articular organs but that they do not initiate a complement cascade there. Mathis suggested that the reason for the joint localization and cascade initiation is the lack of complement inhibitory factors on the joint surface, which, unlike other organ surfaces, does not have an outer cellular layer.

Finally, Mathis proposed a model for initiation and progression of rheumatoid arthritis in K/BxN mice. First, necrotic cells release GPI, which circulates in minute amounts in the bloodstream but accumulates on the joint surface, probably as a result of low-affinity interactions with carbohydrate chains of the cartilage extracellular matrix. Next, autoreactive B cells internalize complexes between B-cell receptors and GPI, and present them on MHC class II molecules to KRN-transgenic T cells. In turn, these T cells stimulate the presenting B cells to produce high levels of anti-GPI IgGs. These IgGs bind to GPI on the joint surface, forming large complexes leading to the activation of the complement system. The complement component C5a functions together with the Fc unit of the autoreactive IgGs to recruit and activate leukocytes and, in particular, neutrophils. These cells eventually invade the joints, resulting in the destruction characteristic of rheumatoid arthritis. It will be interesting to see how this revolutionary revision of the established wisdom - namely that rheumatoid arthritis is a T-cell-mediated disease - will stand up in the human case.

Innate immunity

A large part of the meeting was devoted to innate immunity. In recent years most attention has been focused on cell-surface receptors from the Toll-like receptor (TLR) family (presented at the meeting by Jules Hoffmann, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France and Bruce Beutler, Scripps Research Institute, La Jolla, USA). These proteins are structurally similar to Toll, which is an important receptor involved in natural immunity in *Drosophila*. They are now seen as a family that provide the main recognition sites on many, perhaps most cell surfaces for many products that characterize pathogens (for example, surface LPS, peptidoglycan, flagellin, unmethylated CpG-DNA, and double-stranded RNA). Stimulation of these receptors ultimately leads to activation of the whole programme of immunity.

A highlight was the talk given by Gabriel Nuñez (University of Michigan, Ann Arbor, USA) about a family of cytosolic receptors called Nods. Several members of this family have been found in mammals. They are related to a class of plant resistance genes (*R* genes) that confer resistance to pathogens by inducing several defense programs, such as alterations of host-cell metabolism or cell death. The *R* genes contain an amino-terminal Toll-IL-1-receptor domain (TIR), a central nucleotide-binding domain (NBD) and a carboxy-terminal domain containing multiple leucine-rich repeats (LRRs). Recently, Nuñez and colleagues identified in mammals several proteins with a similar domain architecture containing the central NBD or NOD (nucleotide-binding oligomerization domain) and the amino-terminal LRR domain, but bearing amino-terminal caspase-recruitment domains (CARD) instead of the TIR domain. Two of them, namely Nod1 and Nod2, were most surprisingly shown to behave as cytosolic receptors for bacterial products. Whereas the expression of Nod2 is restricted to monocytes, Nod1 is expressed ubiquitously in adult tissue. Activation by bacterial LPS in the case of Nod1 and Nod2 and by bacterial peptidoglycan in the case of Nod2 leads to activation of NFκB (nuclear factor κB) and subsequent transcription of target genes.

Nuñez showed that the LRR domains are essential for LPS responsiveness, because truncated proteins lacking the LRRs do not mediate LPS-induced activation of NFκB. He presented a model according to which the recognition of LPS (or other unknown activators) by Nods leads to

self-oligomerization and recruitment of RICK, a CARD containing serine/threonine kinase, via CARD-CARD interaction. Subsequently RICK activates NFκB in a way that is dependent on IκB (inhibitor κB) manner. Nuñez reported that, interestingly, this pathway is independent of MyD88 and TRAF6, two essential components of TLR4 signaling. So the TLRs as well as the Nods are receptors sensing pathogen-associated molecular patterns in different environments. They initiate different signaling pathways that merge at the level of NFκB activation.

A frameshift mutation and two point mutations in Nod2 are associated with Crohn's disease, a chronic inflammation of the gut. The frameshift results in a truncated protein that lacks a part of the last LRR of Nod2 and abolishes responsiveness to LPS. Nuñez speculated that lack of Nod2 signaling in monocytes leads to the inability to control enteric bacteria, abnormal T-cell responses and eventually inflammation. He reported that there are over 30 Nod homologs in the human genome, yielding the fascinating possibility that, like the TLRs, these proteins build an intracellular surveillance system for pathogen-associated molecular patterns.

Gene silencing

Just a few years ago a new system was discovered that can protect animals and plants from RNA viruses and transposons. Several terms, such as RNA interference (RNAi), post-transcriptional gene silencing (PTGS), quelling and cosuppression have been used to describe basically the same protection phenomenon. David Baulcombe (The Sainsbury Laboratory, Norwich, UK) presented some fascinating work conducted to reveal the mechanism by which this system works. (I will accept Baulcombe's proposal and generally refer to this phenomenon as gene silencing.) Gene silencing has many features of the adaptive immune system in vertebrates in that it is highly specific, systemic and confers a degree of memory. Strikingly, it seems to be present in all eukaryotes from fungi, plants and invertebrates to vertebrates, including mammals. The fact that, at least in plants, certain viruses produce factors interfering with gene silencing underlines the importance of this system in immunity. Baulcombe and colleagues used *Arabidopsis thaliana* as a model to dissect certain steps involved in the initiation and systemic spread of gene silencing. Plants expressing a green fluorescent protein (GFP) transgene under a constitutive promoter turned out to be particularly useful for this purpose, because systemic spreading of gene silencing could be easily assessed by silencing of GFP fluorescence throughout the plant. By means of either viral infection or delivery of a second transgene by *Agrobacterium* transformation, Baulcombe was able to analyze which genes are involved in transgene- or virus-induced gene silencing. At least two pathways are involved. The first is initiated by transgenes generating single-stranded RNA transcripts that depend on SDE1, an RNA-dependent-RNA-polymerase (RdRP), and SDE3, an RNA helicase that converts single-stranded RNA into double-stranded RNA. In contrast, virus-induced gene silencing is SDE1- and SDE3-independent, because the virus brings its own RdRP.

It was remarkable to see how the exploitation of genomic data contributes to the revelation of fundamental principles of immunity throughout eukaryotes as well as to the discovery of differences between and even within species. For example, the same gene silencing mechanisms and receptors for pathogen-associated molecular patterns containing NBD, TIR, CARD and/or LRR domains are present in both plants and mammals. But Peter Parham (Stanford University School of Medicine, USA) emphasized that there are substantial differences in the natural killer (NK) cell receptor repertoire even between humans and chimpanzees, although their genomes are estimated to be about 98.8% identical. Stephen O'Brien (National Cancer Institute, Frederick, USA) reported that variations in a single gene can have a huge impact on the control of HIV infection and progression to AIDS: 26% of rapid progressors and 20% of long-term survivors bear certain chemokine receptor, killer immunoglobulin-like receptor or HLA alleles, and such people have a difference in life span of up to ten years or more.

It is becoming strikingly clear that immunity is conferred by many, highly interconnected layers of recognition and effector systems. New essential players are being discovered all the time. A good example is a family of GTP-binding proteins, the so-called p47 GTPases, whose indispensable role in mice in providing resistance against intracellular protozoa and bacteria was discovered only in the past two years (presented by Greg Taylor, Duke University, Durham, USA). This complexity, which is common and vital to eukaryotic life, is also reflected on the genomic level and it will be fascinating to see how large the proportion of the genome that is involved in fighting pathogens ultimately proves to be; recent experiments on macrophages have already implicated 25% of the genome in playing this role.

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